THE SEPARATION AND STUDY OF THE ARSENIC CONTAINING CONSTITUENT OF SHRIMP

by

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INTRODUCTION

The presence of arsenic in foods has become a prominent and potential source of study for the chemist in recent years. Whether this arsenic is the natural occurring constituent of plants and animals or that which has accumulated due to various fruit sprays, by-products of manufacturers, and other artificial means makes no difference in the importance of the subject.

Over a century ago when the presence of arsenic in putrid human bodies was first detected, chemists began their study to find whether or not arsenic is a normal constituent of animal organism. They have met with many difficulties in their endeavor to isolate and identify these compounds. Much progress has been made in the last few years on this work, and the chemist may in time enlighten the public to the real importance of arsenic in animal and plant metabolism.

Arsenic in the inorganic compounds usually presents no particular difficulty in the quantitative determination of the amount present. This is also true in case of most of the organic arsenical compounds. There are, however, a few materials where difficulty is presented. Some of the compounds of this type are those contained in shrimp, oils and tobacco.

The results reported in this thesis were obtained in an investigation dealing with the separation and examination of the arsenic containing constituent of shrimp.

HISTORICAL

It has been known for many years that shrimp, lobster, oysters, fish and most types of marine life contain arsenic in varying amounts, but very little is known of the manner in which the arsenic is combined in the tissue. Shrimp in particular contains arsenic in surprisingly large quantities.

The principal source of this arsenic is still open to investigation, but it is known that arsenic is contained in marine algae and in several edible varieties of the seaweeds. Sea water also contains an appreciable quantity of arsenic. The arsenic appears in organic combination in plants and animals and is thought to play a vital part in the activity of the cells. Arsenic expressed as arsenious oxide in the air dried seaweeds is reported as appearing in amounts varying from 6 to 125 parts per million. This follows closely the amounts reported present in the air dried flesh of certain species of marine life.

Coulson, Remington and Lynch (5) have reported that the arsenic contained in shrimp during several seasons and from different localities varied from 1.27 mg. to 171.00 mg. per kg. of the air dried material. They have shown that there

is definitely a seasonal variation in the arsenic content of shrimp. Their feeding experiments upon rats show that the arsenic as it is bound up in shrimp is comparatively non toxic, and does not accumulate in the animal body.

Investigations indicate that this organic arsenical compound which is found in shrimp is not a cacodylate or related compound, but may be and probably is a compound of a simple fat-like series. Some workers believe that the arsenic is bound in a rather complex molecule. Sadolin (10) in one of his reports explains briefly the lecithin, nuclein and keratin theories. In the lecithin theory the arsenic is assumed to be substituted for phosphorous in the molecule. In whatever combination the arsenic appears in shrimp it is present in a compound that is difficult to decompose.

Cassil (3) shows that the arsenic recovered after the ordinary sulfuric acid-nitric acid digestion is less than that recovered by using the same type of digestion followed by co-precipitation of phosphate and arsenate, which in turn is less than that recovered upon treatment with perchloric acid. The amounts recovered after perchloric acid treatment were slightly greater than those obtained after dry ashing methods. This study shows that the perchloric acid treatment makes a very efficient method in converting the arsenic into the inorganic state. In this work the

Gutzeit method of analysis was used in determining the amounts of arsenic in each case.

The fact that Chapman (4) found the arsenical constituent of lobster to be soluble in acetone whereas that in shrimp is not, and due to other variations reported in the properties of the arsenic derivatives, it is fairly well established that the arsenic compounds contained in the several forms of marine life are not exactly the same.

SOURCE AND COMPOSITION OF THE SHRIMP

The material used in this work was ground dried shrimp which was furnished by the United States Bureau of Fisheries. The shrimp were taken in deep water near the mouth of the Stono River on October 29, 1936. This river empties into the Atlantic Ocean along the eastern coast near Charleston, South Carolina. The raw material consisted of around seventy-five pounds of peeled shrimp with their heads and livers removed. They were dried for about sixty hours in porcelain dishes over a steam table, and then for thirty-six hours in an electric oven at 80 to 90 degrees centigrade, then ground through a burr mill. The size of the ground material ranged from a fine meal to particles measuring about one-eighth inch across. The tissue was light tan to brown in color and had an unpleasant odor.

Analysis of the raw material gave the following results:

	Lot 1	Lot 2
Arsenic (as As_2O_3)	122.5 mg./kg.	131.25 mg./kg.
Moisture	2.38 percent	2.38 percent
Nitrogen	14.20 "	----
Ash	7.00 "	7.00 percent

Qualitative analysis of the ash showed the presence of phosphorous, chlorine, carbon dioxide, magnesium, calcium, aluminum, iron and free silicon dioxide. Lots one and two in the above table were taken from the same allotment of shrimp tissue, but lot one contained a greater amount of the coarse material. This coarse material was composed mostly of legs of the shell fish.

EXPERIMENTAL

Separation of the Arsenic Containing Constituent

If the arsenic containing constituent of shrimp is a lecithin type of compound as suggested by Sadolin (10) then it would be insoluble in acetone and possibly other solvents, but soluble in alcohol. The most practical method for separating the arsenic containing constituent of shrimp therefore would be by the use of solvents. Extraction methods of separating the arsenic bearing constituent were tried and found to be satisfactory. Extractions were made (a) by digestion extraction, (b) by continuous flow of sol-

vent type of extraction and (c) by the intermittent or soxhlet type of extraction.

Digestion Extraction. The following digestion method of extracting the arsenic containing constituent was suggested in a confidential report by Coulson[1].

One hundred grams of the dry ground shrimp containing 122.5 milligrams of arsenic (as arsenious oxide) per kilogram were placed in a glass jar and covered with 600 cc. of 85 percent ethyl alcohol. The jar was placed in a water bath and the contents heated to boiling. The alcohol was then allowed to cool and stand over the shrimp tissue. After standing for two days the alcohol solution was filtered and the residue washed twice with 50 cc. portions of 95 percent ethyl alcohol. Analysis of an aliquot of the combined filtrates showed that 58 percent of the arsenic had been recovered.

This did not make a satisfactory method of extraction due to the fact that only a little more than one-half of the arsenic containing constituent was recovered.

Extraction by Means of the Soxhlet Extractor. The arsenic containing constituent of shrimp was found to be insoluble in acetone and diethyl ether. Therefore it was proposed to extract by means of a soxhlet type of extractor,

[1]Coulson, E. J. Further experiments upon the nature of the naturally occurring arsenic compound of shrimp. Confidential report.

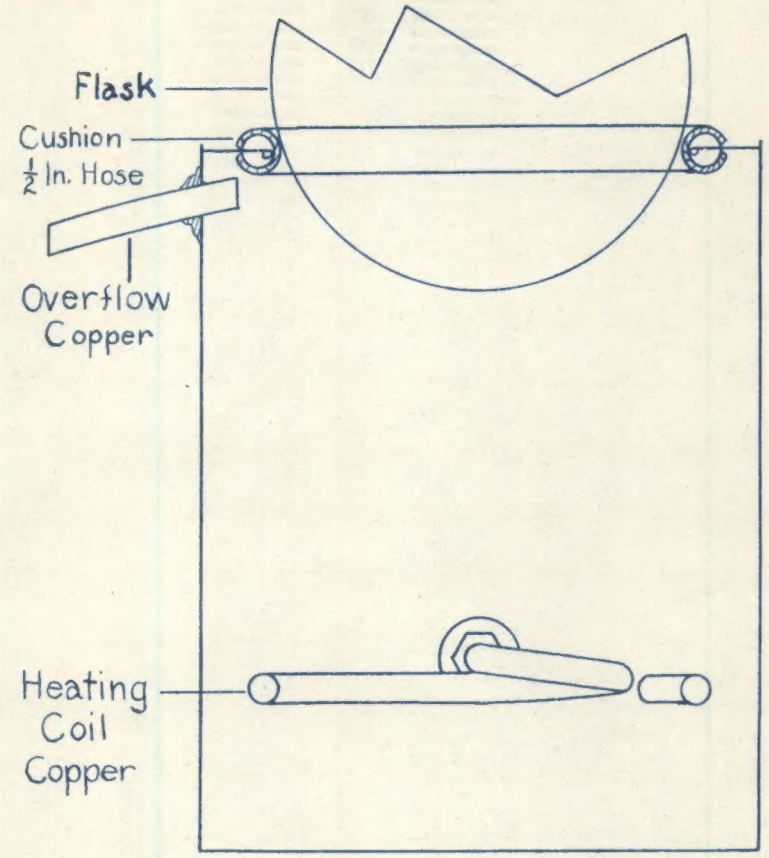
the dried material first with acetone and second with diethyl ether to remove substances soluble in these solvents, and then to extract the residue with solvents which would dissolve the arsenic containing constituent. A water bath (Plate 1) was used in heating the solvents. This plan was used in the following procedure.

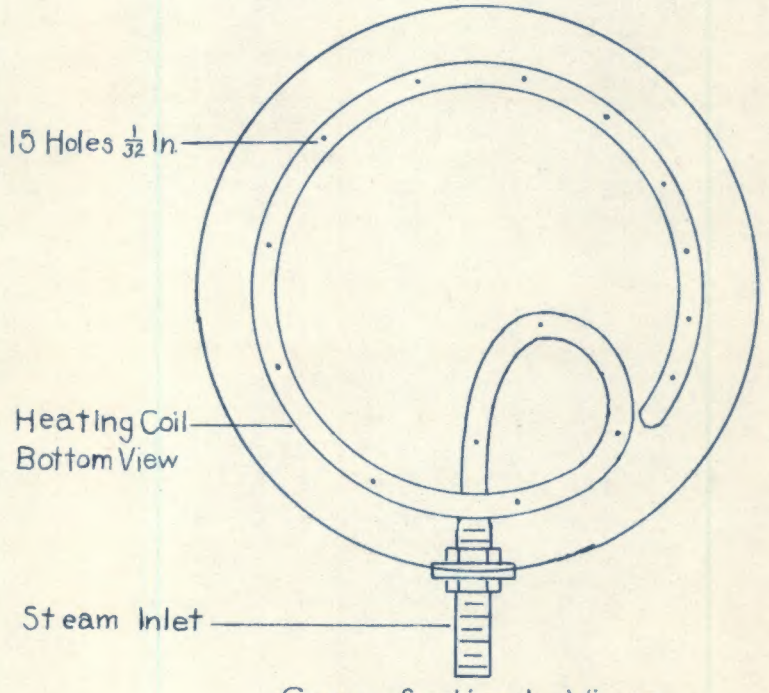
A sample of shrimp tissue was placed in a small soxhlet extractor and extractions made as follows:

18.00 g. of dried shrimp tissue were extracted with 250 cc. of acetone until the filtrate became colorless.

- Residue: Extracted with 250 cc. of diethyl ether until the filtrate became colorless.
  - Residue: Extracted with 250 cc. of 85 percent ethyl alcohol until the filtrate became colorless.
    - Residue: Contained 4 percent of the arsenic.
    - Extract: Contained 95 percent of the arsenic.
  - Extract: No arsenic was detected.
- Extract: Contained a trace of arsenic.

PLATE I





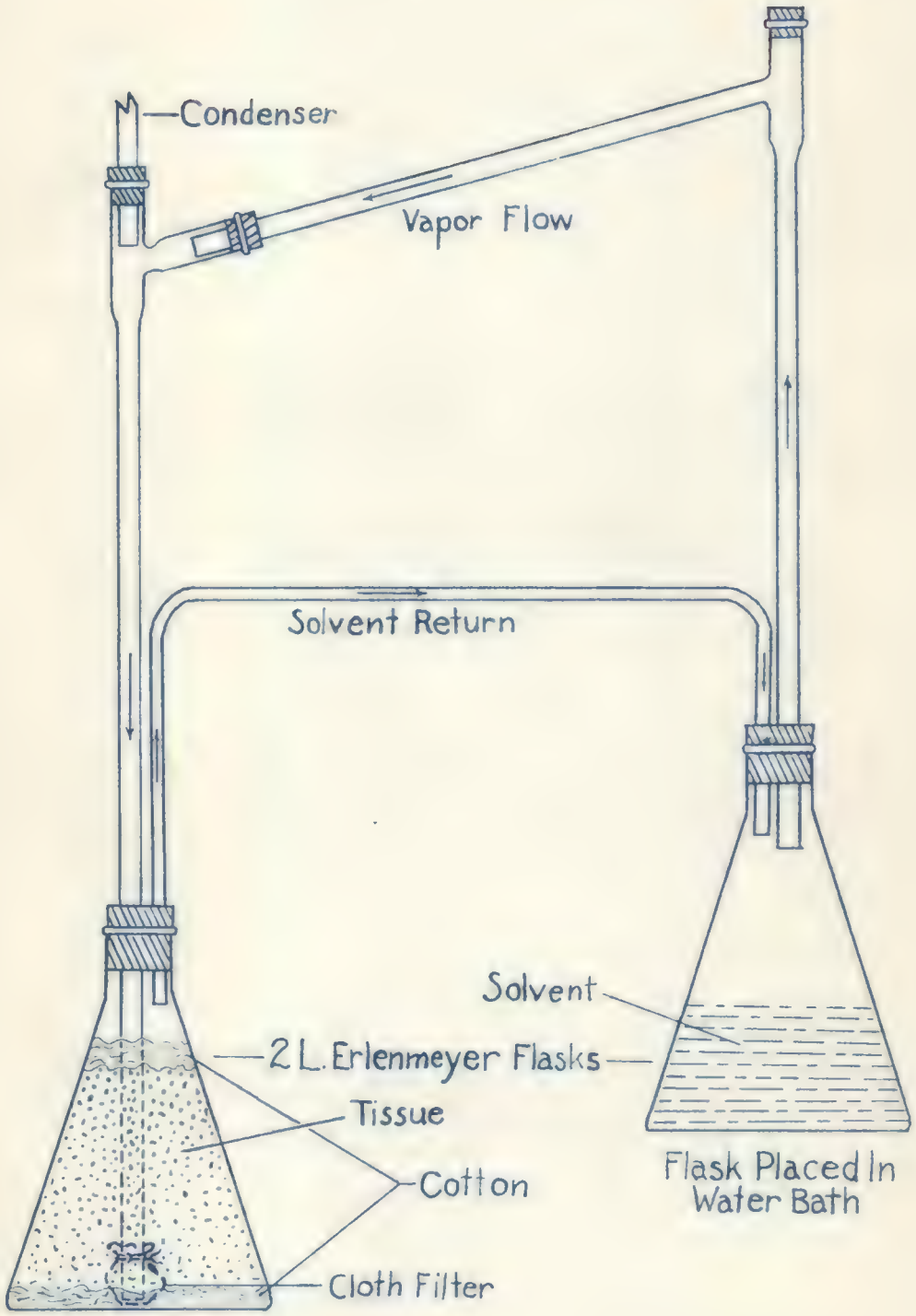
Cross Sectional Views
Scale ¼ In = 1 In.

WATER BATH

Another sample of shrimp tissue was extracted in the same extractor using identical amounts of materials with the following exception. In place of 95 percent ethyl alcohol, 250 cc. of absolute methyl alcohol were used to extract the arsenic containing constituent. In this case 96.6 percent of the arsenic was recovered in the solvent and 3.4 percent was retained in the residue. Absolute methyl alcohol was substituted for the 95 percent ethyl alcohol to establish a good solvent for recovering the arsenical compound that would keep it as free as possible from contact with water. It was next proposed to extract from the original shrimp tissue the arsenic bearing constituent with absolute methyl alcohol to establish just how much of the arsenic could be recovered with this solvent. Analysis showed that 96.4 percent of the arsenic had been recovered. As has been shown the soxhlet extractor makes a very efficient extractor for securing the arsenic containing constituent.

Continuous Flow Extraction. Although the soxhlet extractor made a very convenient and efficient piece of apparatus for obtaining the arsenic compound of shrimp, it was proposed to employ the continuous flow or solvent type of extractor (see Plate 2) for a part of this investigation. It was thought that if this type of extraction proved to be satisfactory, then an extractor that could be made in any

PLATE II



CONTINUOUS FLOW EXTRACTOR

laboratory for about one-tenth the cost of the soxhlet extractor would be available to other investigators.

The following procedure was carried out in this work. Eighty-five percent ethyl alcohol was used in place of absolute methyl alcohol in extracting the arsenic containing constituent because it was found that more of the arsenic could be precipitated from this solvent upon the addition of acetone.

606 g. of dried shrimp were extracted in the continuous flow extractor (see Plate 2) with 3200 cc. of acetone until the solution standing over the material had become clear.

- Residue: Extracted with 2250 cc. of diethyl ether until the solvent standing over the material became clear.
  - Residue: Extracted with 2100 cc. of 85 percent ethyl alcohol until a clear solution stood over the tissue.
    - Residue: Contained 12.36 percent of the arsenic.
    - Extract: Contained 24.06 percent of the arsenic.
  - Extract: No arsenic was detected.
- Extract: Traces of arsenic were detected.

This proved to be a fairly efficient means of extracting the arsenic containing constituent of shrimp, but due to

the inconvenience in manipulation and the greater efficiency of the soxhlet extractor this method was abandoned on further extractions.

Examination of the Arsenic Containing Constituent

Acetone Precipitation of the Arsenic. It was found that the addition of acetone to alcohol extracts of the arsenic bearing constituent would precipitate small quantities of the arsenic from the solution. The amount of the arsenic bearing compound obtained depended upon the amount of acetone added and the concentration of the solution containing the arsenic. The greatest percent of the arsenic could be recovered from the 95 percent ethyl alcohol extract. This method of separation of the arsenic bearing constituent was used in the following work.

The Arsenic Containing Constituent Is Non-Protein. A quantity of absolute alcohol extract from the original shrimp tissue was obtained so that the following study of the arsenic bearing constituent could be made. The soxhlet type of extractor was used in securing the material for this work. The barrel of the extractor had a capacity of about two liters and would handle conveniently a sample consisting of six to eight hundred grams of the dried shrimp tissue.

An aliquot of the extract was evaporated to a brown

viscous liquid under a pressure of 50 to 60 mm. of mercury and at a temperature of 30 to 40 degrees. An equal volume of acetone was added and the material allowed to stand for thirty minutes. A precipitate formed which was of a light cream color and almost a solid. The solution was filtered and the precipitate soon became a brown syrup-like material. This syrup-like material was washed with four 15 cc. portions of acetone, then dissolved in an equal volume of water, and the water solution extracted three times with equal volumes of diethyl ether. The ether soluble portion was separated and found to contain only a trace of arsenic. The water soluble portion was made up to 100 cc. with water and 75 cc. of the solution was acidified with hydrochloric acid. The acid solution was divided into four equal portions. To two of these were added equal volumes of a saturated solution of picric acid. Equal volumes of 5 percent trichlor-acetic acid were added to the other two. Protein precipitates were formed in both cases. Analysis of the precipitates showed that no arsenic had been precipitated, thus establishing that the arsenic bearing constituent is not a protein. The filtrates were placed together, evaporated to a small volume and analyzed for arsenic. Eighteen milligrams of arsenic as arsenious oxide were recovered. This analysis was made to show that the arsenic was still in solution.

The remaining 25 cc. of the water soluble portion was divided and equal volumes of N sodium chloride solution added to each. The solutions were then acidified with acetic acid (to phenolphthalein) and heated to boiling. Protein precipitates were formed which did not contain arsenic. This study further indicates that the arsenic bearing constituent is not a protein.

Hydrolysis of the Arsenic Bearing Constituent. Aliquots of absolute methyl alcohol extract of the original shrimp tissue were evaporated to near dryness over a water bath. The residue obtained was hydrolysed with an alcoholic solution of sodium hydroxide (100 cc. 95 percent alcohol and 20 cc. of 40 percent sodium hydroxide) by refluxing over a water bath for about four hours. After four hours of refluxing the solution was evaporated to a small volume to remove most of the alcohol. This left a solid mass when the solution had cooled. The solid material was heated with 12 volumes of water acidified with hydrochloric acid, until a brown, oily layer of fatty acids separated. The water layer was separated and the oily layer washed twice with water, the washings being added to the water solution. The arsenic was removed from the water solution by co-precipitation of magnesium ammonium phosphate and arsenate (6). Quantitative analysis of the arsenic obtained showed that from 75 to 98

percent of the arsenic could be recovered in this manner. Hydrolysis was tried by the same procedure using 40 percent aqueous sodium hydroxide in place of the alcoholic solution. Analysis of the arsenic showed that most of the arsenic had been recovered in the inorganic state.

This study indicates that the arsenic containing constituent in shrimp is difficultly decomposed by hydrolysis.

Concentration and Properties of the Arsenic Containing Constituent. An aliquot of 85 percent ethyl alcohol extract of shrimp tissue (tissue that had been extracted first with acetone and second with diethyl ether) was allowed to stand with one and one-tenth volumes of absolute methyl alcohol for thirty minutes. The mixture was then centrifuged to remove a white, flake-like precipitate that had formed. The precipitate was discarded. Analysis of an aliquot of the methyl alcohol-ethyl alcohol solution showed that it contained approximately 100 percent of the arsenic. To this alcohol solution were added two volumes of acetone and the mixture allowed to stand thirty minutes. A light tan, taffy-like precipitate formed. The precipitate was removed by centrifuging the solution. Analysis showed that the precipitate contained 11.12 percent of the arsenic.

Twenty volumes of absolute ethyl alcohol were added to the arsenic containing precipitate and the mixture heated

in a water bath (just brought to boiling) with stirring for one minute and the solution decanted. The decanted ethyl alcohol solution was evaporated over a water bath until a white, flake-like precipitate just began to settle out. The remainder of the alcohol was removed in a vacuum desiccator over calcium chloride. The precipitate obtained was a white mass resembling beef tallow in appearance. This white material rapidly became a brown, syrup-like mass when exposed to the air. The brown mass was dissolved in absolute ethyl alcohol and reprecipitated with two volumes of acetone. The precipitate came down as a white, meal-like material and was separated by centrifuging the solution. This precipitate was dissolved in the least amount of absolute ethyl alcohol and placed in the vacuum desiccator. As the alcohol evaporated the same white, flake-like material began to settle out and when dry the precipitate resembled beef tallow in appearance. When a drop of water was added to this material it immediately became a brown, gelatinous mass. The white material was found to contain 9.56 percent arsenic, and gave tests for nitrogen and traces of phosphorous. The nitrogen was evolved as ammonia from this white material when warmed with sodium hydroxide. This information indicates that the arsenic containing compound could be a fat-like compound, possibly a kephalin.

Since the material readily took up water and moisture from the air it was decided to try and obtain the arsenic containing constituent by use of absolute methyl and ethyl alcohols. The following procedure was carried out in this study.

Six hundred twenty-five grams of the dried shrimp tissue were extracted in a large soxhlet extractor with 2250 cc. of absolute methyl alcohol. The extraction was allowed to proceed for five hours after the solution that stood over the tissue had cleared. Analysis of an aliquot of the extract showed that 90.4 percent of the arsenic had been recovered.

Three volumes of absolute ethyl alcohol were added to the absolute methyl alcohol extract in the following manner: An equal volume of the alcohol was added and allowed to stand fifteen minutes. A white, flake-like precipitate was formed and was separated by filtration. The second portion of alcohol was added and allowed to stand for fifteen minutes. More of the white precipitate was formed and removed by filtration. The last addition of ethyl alcohol was now made and the mixture allowed to stand for 36 hours. The white precipitate formed more slowly after the last addition of absolute ethyl alcohol. The precipitate was a white, fatty material which adhered to the flask.

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The methyl alcohol-ethyl alcohol solution of the arsenic containing constituent was filtered and evaporated to approximately one-sixth volume at a pressure of 50 to 60 mm. of mercury and at a temperature ranging from 30 to 40 degrees. More of the white, fatty precipitate was formed as the alcohol was distilled. The residual solution was filtered and found to contain 90.25 percent of the arsenic. Two volumes of acetone were added to this condensed solution, allowed to stand fifteen minutes and centrifuged. The precipitate consisted of a mixture of white, meal-like material and a brown, taffy-like substance. The precipitate contained about 9 percent of the arsenic.

Twenty volumes of absolute ethyl alcohol were added to the precipitate just obtained. The mixture was placed in a water bath just brought to boiling and stirred for two minutes and the ethyl alcohol solution decanted. The ethyl alcohol solution thus obtained was found to contain 6.2 percent of the arsenic. The alcohol was removed by evaporation over a water bath to a small volume and the last traces of alcohol removed in a vacuum desiccator. The dried material was a light tan, fat-like substance. This material was taken up in a small amount of absolute ethyl alcohol, placed in a vacuum desiccator and evaporated to dryness. As the solution evaporated a white, flake-like precipitate began to

form. When dry the substance was white and resembled beef tallow in appearance.

The white material thus obtained was analyzed and found to contain 9.1 percent arsenic. Qualitative analysis proved the presence of nitrogen and phosphorous. The nitrogen was evolved from the material in the form of ammonia when warmed with sodium hydroxide. This indicates that the arsenical compound could be a kephalin if it belongs to this series of compounds with arsenic replacing phosphorous in the molecule.

The arsenic containing constituent obtained by this method seemed to have the same physical and chemical properties as those obtained in the preceding study. The substance would change to a brown, gum-like material when exposed to the air or when a small amount of water was added. When the brown, gum-like material was taken up in absolute ethyl alcohol and reprecipitated with acetone a white, meal-like substance was obtained. The addition of absolute ethyl alcohol to either the white, meal-like substance or the brown, gum-like material, and the subsequent drying in a vacuum desiccator over calcium chloride produced the same white, tallow-like compound.

Method of Analysis

The method of analysis used for the quantitative determination of arsenic in samples used in this report was taken from "Methods of Analysis" (1, p. 573). The use of ammonium oxalate-urea solution for the elimination of oxides of nitrogen was found to be unnecessary in the preparation of the sample.

Disintegration of the Arsenic Containing Material. The material to be analysed is placed in an 800 cc. kjeldahl flask and then a mixture of 15 cc. concentrated nitric acid and 20 cc. concentrated sulphuric acid are added cautiously. If the sample is dry, it is necessary to wet the material with 10 to 15 cc. of water. Twenty cc. is all the sulphuric acid that is used during the entire process of digestion and is also the amount used in the blank. It is necessary to make the addition of the mixture of acids slowly and cautiously to prevent "foaming over" of the sample. After the first rather violent reaction has slowed down the mixture is heated gently until it turns a dark brown in color. Fifteen cc. of nitric acid are now added and the solution again heated until it becomes a dark brown in color. Nitric acid is added in 15 cc. portions in like manner until the solution becomes clear and remains clear upon heating to dense,

white fumes of sulphur trioxide. If the solution should have a pale yellow cast after heating to dense fumes of sulphur trioxide it is usually unnecessary to repeat the addition of nitric acid. The solution is then cooled to room temperature and 9 cc. of perchloric acid 70 percent or 10 cc. 60 percent are added. The solution is then heated gently (so that it just will boil) for 30 to 40 minutes, and then heated over a strong hot flame for 8 to 15 minutes. If the solution is not clear after the perchloric acid treatment 15 cc. of concentrated nitric acid are added and the solution heated to dense, white fumes of sulphur trioxide. The perchloric acid treatment is then repeated and the sample is ready for isolation and determination of the arsenic.

Separation and Determination of the Arsenic. The arsenic is found in the reaction mixture which is prepared in this way as meta-arsenic acid. It is liberated from the mixture as arsenious chloride and is carried over by distillation with hydrogen chloride gas. The reaction mixture is treated with hydrazine sulfate to reduce the arsenic to the trivalent state, and with sodium chloride to react with sulphuric acid and give the hydrogen chloride gas. The principal reactions taking place in recovering the arsenic are represented as follows:

$2\ NaCl + H_2SO_4 \longrightarrow 2\ HCl + Na_2SO_4$

$N_2H_4 \cdot H_2SO_4 + 2\ HAsO_2 + 6\ HCl \longrightarrow 2\ AsCl_3 + N_2 + 6\ H_2O + H_2SO_4$

The amount of arsenious chloride is determined by titrating the distillate with a standard potassium bromate solution. Methyl orange is used as the indicator, and its color is destroyed by the potassium bromate at the end point. The reaction taking place in this titration is shown by the following equation:

$KBrO_3 + 3\ AsCl_3 + 6\ H_2O \longrightarrow 3\ HAsO_2 + 9\ HCl + KBr$

DISCUSSION

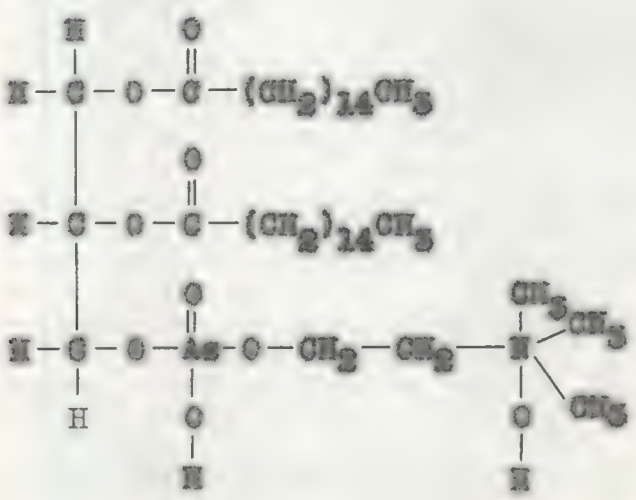
The arsenic as it is found in shrimp is not present in the inorganic state, but is bound up in some complex organic molecule. The results obtained in this work point to the fact that the arsenic of shrimp is present in some non-protein compound that is not broken down completely by the action of hot mixtures of concentrated sulphuric and nitric acids.

Compounds in which arsenic is joined directly to carbon are difficult to decompose by ordinary methods of oxidation. The cacodylates belong to this class of compounds. The arsenic of shrimp cannot be bound up in a compound of this class because the cacodylates are soluble in chloroform,

whereas the natural occurring arsenic compound of shrimp is not.

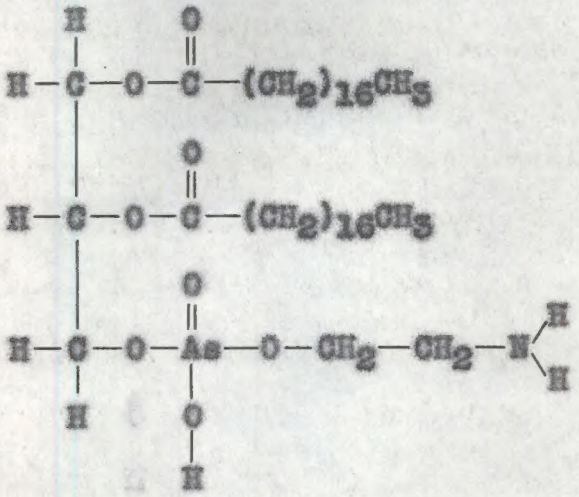
Further facts pointing to the complexity of the arsenic containing molecule in shrimp were evidenced when it was found that the arsenic could be hydrolyzed into the inorganic state by concentrated aqueous and alcoholic solutions of sodium hydroxide. The arsenic bearing constituent as it was obtained gave qualitative tests for nitrogen and phosphorous. The nitrogen was evolved as ammonia when the arsenic containing constituent was warmed with alkali. If this ammonia were obtained from the arsenical molecule then the arsenic compound could be a kephalin. From the results obtained in this work it is thought that the arsenic in shrimp is a fat-like compound and is possibly a lecithin or a kephalin.

A lecithin-like compound in which arsenic replaces phosphorous of the formula



would contain 9.43 percent arsenic. This approaches the amount actually found in the arsenic containing material isolated.

The kephalin type of compound in which phosphorous is replaced by arsenic represented by the formula



contains 9.48 percent arsenic. This is approximately the same percentage composition of arsenic as that of the arsenic constituent isolated from shrimp.

SUMMARY

The arsenic compound in shrimp is soluble in, and can be extracted by absolute methyl alcohol, absolute ethyl alcohol and water solutions of both ethyl and methyl alcohols.

Acetone was found to extract noticeable traces of the arsenic containing constituent in shrimp. Diethyl ether

extraction of the shrimp gave no evidence showing that any of the arsenic compound had been extracted.

The arsenic as it occurs in shrimp is present in a more or less complex organic molecule which cannot be completely decomposed by the action of hot concentrated sulphuric and nitric acids, but is hydrolyzed by aqueous and alcoholic solutions of sodium hydroxide.

The arsenic containing constituent is precipitated from solutions in certain organic solvents upon the addition of acetone.

ACKNOWLEDGMENT

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Appreciation is also due Mr. Lawrence Taylor who aided in this work for one semester in a senior problem.

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